HEART RATE VARIABILITY IN CIRRHOSIS LIVER

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CERTIFICATE

This is to certify that the dissertation entitled **"**HEART RATE VARIABILITY IN CIRRHOSIS LIVER**"** is the bonafide original work of **Dr. S. ARAVINDH** in partial fulfillment of the requirements for **D.M (GASTROENTEROLOGY) BRANCH – IV** Examination of the Tamilnadu Dr. M.G.R. Medical University to be held in August 2009. The period of study was from June 2007 to December 2008.

Dr. J. MOHANASUNDARAM, M.D., Ph.D, DNB,
DEAN
Govt. Stanley Medical College & Hospital,
Chennai-600 001.

Prof. V. JAYANTHI , M.D.,D.M,
Professor & Head
Department of Medical Gastroenterology
Govt. Stanley Medical College
Chennai-600 001.

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Introduction

Hyperdynamic circulatory state is a common and long-recognized feature of patients with decompensated chronic liver disease (1). It is characterized by elevated cardiac rate and output and reduced peripheral vascular resistance, with pooling of blood in the splanchnic and peripheral circulation and effective central hypovolemia.

The autonomic nervous system plays a central role in modulating cardiac performance and the vasomotor activity in the hyperdynamic circulatory state (2).

The presence of an autonomic dysfunction (AD) in cirrhosis has been clearly shown through different experimental approaches, including the evaluation of the cardiovascular and sudomotor responses to physiological and pharmacological stimulation (3-6), and by showing a hyperproduction of weak adrenergic neurotransmitters (7).

It has also been reported that the severity of AD is proportional to the severity of cirrhosis (5,8), and its presence is an indicator of poor prognosis in patients with both early and advanced liver disease (9,10).

In an earlier study from our department, the presence of AD correlated with the occurrence of variceal bleed (11).

Heart rate variability implies variations in the interval between consecutive heart beats as well as between consecutive instantaneous heart rate, ie, it describes the variations of both instantaneous heart rate and RR intervals (12). It is a simple test to assess the function of the autonomic nervous system and its modulation on the heart rate.

Measurement of AD in chronic liver disease, using Heart rate variability (HRV), has been shown to correlate with the underlying severity of the liver disease (13). But, there are no studies from India, on the impact of Heart rate variability in predicting the severity, prognosis and bleed pattern in patients with cirrhosis liver.

Aim of the study

- To assess Heart rate variability (HRV) as a test for Autonomic nervous system (ANS) function in patients with liver cirrhosis, comparing it with healthy controls.
- To correlate HRV results with the severity of the underlying liver disease
- To compare HRV outcome between
  - Bleeders and non-bleeders
  - Survivors and non-survivors

Review of literature

Autonomic Nervous System (ANS)

The ANS is predominantly an efferent system transmitting impulses from the central nervous system (CNS) to peripheral organs. Its effects include control of Heart rate (HR) and force of heart contraction, constriction and dilatation of blood vessels, contraction and relaxation of smooth muscle in various organs, and glandular secretions. Autonomic nerves constitute all of the efferent fibers that leave the CNS, except for those that innervate skeletal muscle (14, 15). There are some afferent autonomic fibers (ie, from the periphery to the CNS) that innervate the baroreceptors and chemoreceptors in the carotid sinus and aortic arch, which are important in the control of HR, blood pressure, and respiratory activity. The ANS is divided into two separate divisions, parasympathetic and sympathetic, based on anatomical and functional differences (Table 1).

Parasympathetic Nervous System

The preganglionic outflow of the parasympathetic nervous system (PNS) arises from the brain stem and is known as the craniosacral outflow. The vagus nerve (or Xth cranial nerve) carries fibers to the heart and lungs (as well as other organs) and is the primary parasympathetic innervation of these organs. The PNS is largely concerned with conservation and restoration of energy by causing a reduction in HR and blood pressure and by facilitating digestion and absorption of nutrients and discharge of wastes. The chemical transmitter at synapses in the PNS is acetylcholine (Ach); thus, nerve fibers that release Ach from their endings are described as cholinergic. The specific Ach receptors have been further subdivided pharmacologically by the actions of the alkaloids muscarine and nicotine on these receptors. Postganglionic parasympathetic nerve endings, the response of which to Ach is mimicked by muscarine, are referred to as muscarinic Ach receptors, and postganglionic receptors, the response of which to Ach is mimicked by nicotine, are termed nicotinic Ach receptors. Vagal tone declines with aging, and the only physiological stimulus that has been found to increase vagal tone is regular

dynamic exercise.

Table 1: Functional consequences of autonomic innervation

Physiologic function	Sympathetic	Parasympathetic
Heart rate	Increased	Decreased
Blood pressure	Increased	Mildly decreased
Bladder	Increased sphincter tone	Voiding (decreased tone)
Bowel motility	Decreased motility	Increased
Lung	Bronchodilatation	Bronchoconstriction
Sweat glands	Sweating	-
Pupils	Dilatation	Constriction
Adrenal glands	Catecholamine release	-
Sexual function	Ejaculation, orgasm	Erection

Sympathetic Nervous System

The cell bodies of the sympathetic preganglionic fibers are in the lateral horns of spinal segments T1 through L2, which comprise the thoracolumbar outflow of the sympathetic ganglionic chains. The adrenal medulla is innervated by preganglionic fibers, and therefore, adrenaline is released from the gland by stimulation of nicotinic Ach receptors. At most postganglionic sympathetic endings, the chemical transmitter is noradrenaline, which is present in the presynaptic terminal as well as in the adrenal medulla. The synthesis and storage of the catecholamines adrenaline and noradrenaline (which are synthesized from the essential amino acid phenylalanine) in the adrenal medulla is similar to that of sympathetic postganglionic nerve endings, but most noradrenaline in the adrenal medulla is converted to adrenaline. The adrenal medulla responds to nervous impulses in the sympathetic cholinergic preganglionic fibers by hormonal secretion. In situations involving physical or psychological stress, much larger quantities are released. In contrast to the parasympathetic system, the sympathetic system enables the body to respond to challenges to survival (fight or flight) or situations of hemodynamic collapse or respiratory failure. Sympathetic responses include an increase in HR, blood pressure, and cardiac output; a diversion of blood flow from the skin and splanchnic vessels to those supplying skeletal muscle; bronchiolar dilation; and a decline in metabolic activity. The actions of catecholamines

are mediated by α and ß receptors. ß1-adrenoceptor–mediated effects in the heart, which include increased force and rate of contraction, are differentiated from those producing smooth muscle relaxation in the bronchi and blood vessels, which are ß2-mediated effects.

Hemodynamic changes in Chronic liver disease

Hyperdynamic circulation

Hyperdynamic circulation is a feature of patients with advanced cirrhosis, consisting of elevated cardiac rate and output and reduced peripheral vascular resistance, so that arterial pressure is tendentially or frankly reduced (1,16).

An increase in cardiac output can be attributed to an increase in venous return, heart rate and myocardial contractility, all of which are controlled by the autonomic nervous system. Vasodilatation (low systemic vascular resistance), the presence of arteriovenous communications, expanded blood volume and increased sympathetic nervous activity may further raise the cardiac output; most of these pathophysiological mechanisms are active in advanced cirrhosis (16). In the early stages, the presence of a hyperdynamic circulation is often not apparent. However, with the progression of the liver disease, there is an overall association between the severity of the cirrhosis and the degree of hyperdynamic circulation. Studies on circulatory changes with posture suggest that the patients are mostly hyperdynamic in the supine position (2).

Blood and plasma volumes are raised in advanced cirrhosis, but the distribution between central and non-central vascular areas is unequal (17). Thus, by different techniques it has been established that the central and arterial blood volume- that is, the blood volume in the heart, lungs and central arterial tree, is most often decreased, whereas the non-central blood volume, in particular the splanchnic blood volume, is increased in animals and patients with cirrhosis (Table 2) (16-18). The effective arterial blood volume (ie, the circulatory compartment sensed by baroreceptors) and the central circulation time (ie, central blood volume relative to cardiac output) are substantially reduced and bear a significant

relationship to poorer survival in advanced cirrhosis (19).

Total vascular compliance as well as arterial compliance (ie, an increase in intravascular volume relative to an increase in transmural blood pressure) are increased in cirrhosis with the degree of decompensation (20).

Pathophysiology of splanchnic arteriolar vasodilatation

Arteriolar vasodilatation in cirrhosis and portal hypertension may be brought about by a combination of overproduction of circulating vasodilators, vasodilators of intestinal or systemic origin, vasodilators that escape degradation in the diseased liver or bypass the liver through portosystemic collaterals, reduced resistance to vasoconstrictors and increased sensitivity to vasodilators (16).

According to **''the arterial vasodilation hypothesis''**, splanchnic arteriolar vasodilation leads to reduction of the systemic vascular resistance, central arterial underfilling with effective hypovolaemia, activation of vasoconstrictor systems, such as the sympathetic nervous system (SNS), the renin–angiotensin–aldosterone system (RAAS), vasopressin, endothelins (ETs) and neuropeptide Y (20).

This leads to the development of a hyperkinetic circulatory state. The predominantly splanchnic vasodilation in cirrhosis precedes the increase in cardiac output and heart rate With the progression of the disease, the splanchnic vasodilatation becomes more pronounced and the hyperdynamic circulation may no longer be sufficient to correct the effective hypovolaemia (21). The splanchnic circulation is less sensitive to the effects of angiotensin II, noradrenaline and vasopressin because of the surplus of vasodilators which may play a role in the development of the vascular hyporesponsiveness to vasoconstrictors (22). The arterial blood pressure is mainly maintained by vasoconstriction in the renal, cerebral and hepatic vascular beds; where there seems to be a diminished release of nitric oxide (NO) from endothelial cells (23). To explain the vasodilatation in the systemic circulation, recent research has focused especially on substances such as NO, CGRP and adrenomedullin, but natriuretic peptides, interleukins, hydrogen sulphide, ETs and endocannabinoids have also been implicated (20). Systemic NO production is increased and precedes the development of the hyperdynamic circulation in cirrhosis,

thereby playing a major role in the arteriolar and splanchnic

Table 2: Circulatory changes in specific vascular beds in cirrhosis

Systemic circulation	Changes
Plasma volume	↑
Total blood volume	↑
Non-central blood volume	↑
Central and arterial blood volume	↓
Arterial blood pressure	↓
Systemic vascular resistance	↓
Heart	
Heart rate	↑
Cardiac output	
Left atrial volume	↑
Left ventricular volume	→
Total vascular compliance	↑
Arterial compliance	↑
Renal circulation	
Renal blood flow	↓
Glomerular filtration rate	↓ →
Hepatic and splanchnic circulation	
Hepatic blood flow	↓ →
Hepatic venous pressure gradient	↑
Postsinusoidal resistance	↑

vasodilation and vascular hyporeactivity (23).

Thus, the excess of vasodilators combined with an inadequate haemodynamic response to vasoconstrictors may explain the vasodilatatory state and vascular hyporeactivity in cirrhosis combined with a hyperdynamic circulation, but the pathophysiological mechanisms behind the development of the hyperdynamic circulation in cirrhosis may be multifarious (Table 3).

Hepatic circulation

In healthy subjects, the hepatic blood flow equals the splanchnic blood flow, but patients with portal hypertension have a substantial portosystemic collateral circulation, and an increased mesenteric inflow of up to several litres per minute has been reported (table 2). Thus, a large part of the increased cardiac output is returned through portosystemic collaterals. The azygous blood flow is especially important, as the azygous vein drains oesophageal varices and an increase in azygous flow is associated with an

increased risk of variceal bleeding. there seems to be a defective sinusoidal eNOS-derived production of

NO. In addition, recent investigations of endogenous vasoactive substances have focused especially on ET-1, angiotensin II, catecholamines and leukotrienes in the increased hepatic-sinusoidal resistance (22). The haemodynamic imbalance with a predominant sinusoidal constriction may contribute significantly to the development of portal hypertension

Volume distribution and circulatory dysfunction

Imbalance between vasodilating and vasoconstricting forces in cirrhosis contributes to an abnormal distribution of volume, vascular resistance and flow. Although the cardiac output is increased, thereby reflecting substantial vasodilatation, it covers hyperperfused, **Table 3: Pathophysiological components in the hyperdynamic circulation and cardiovascular dysfunction in cirrhosis**

- Peripheral and splanchnic arterial vasodilatation
    - Baroreceptor-induced increase in heart rate
- Autonomic dysfunction
    - Increased sympathetic nervous activity
    - Vagal impairment
- Alterations in cardiac preload
    - Increased portosystemic shunting
    - Increased blood volume
    - Effects of posture
    - Decreased blood viscosity
- Alterations in oxygen exchange
    - Anaemia
    - Hypoxaemia

- Hepatopulmonary syndrome
- Portopulmonary hypertension

normoperfused and hypoperfused vascular beds. Thus, in the kidney, vasoconstriction prevails and plays a pivotal role along with the development of hepatic decompensation.

Liver dysfunction, central hypovolaemia, arterial hypotension and neurohumoral activation of particularly the RAAS and SNS with renal vasoconstriction is of major importance (20, 21). The increased plasma volume in cirrhosis should therefore be considered secondary to the activation of neurohumoral mechanisms consequent on mainly splanchnic vasodilatation, low arterial blood pressure and reduced central and arterial blood volume (Table 3).

Central hypovolaemia and arterial hypotension may be ameliorated by infusion of plasma expanders. Irrespective of severity, volume expansion produces a rise in stroke volume and cardiac output. In early cirrhosis there is a proportional expansion of the central and noncentral parts of the blood volume, whereas in late cirrhosis, expansion is mainly confined to the noncentral part, with a proportionally smaller increase in cardiac output, probably because of cardiac dysfunction and abnormal vascular compliance (20).

When therapeutic paracentesis is done in decompensated cirrhosis without administration of plasma expanders, about 75% of patients will develop what is termed paracentesis induced circulatory dysfunction. This condition is characterized by a pronounced activation of the RAAS and SNS, which reflects central hypovolaemia. It is mainly caused by a paracentesis-induced splanchnic arteriolar vasodilatation and brings about a further reduction in the systemic vascular resistance. Intravenous infusion of albumin has been shown to prevent complications caused by circulatory dysfunction and may prevent development of renal failure and rapid occurrence of ascites, and prolong survival (24). Recent studies have shown, however, that administration of vasoconstrictors such as terlipressin or noradrenaline may be effective alone or especially in combination with albumin. Paracentesis-induced circulatory dysfunction is thus an example of a cirrhotic condition where complications attributable to a

potentially reduced effective blood volume can be prevented by a specific volume expansion.

The deterioration of the liver function is followed by a decreased renal blood flow and glomerular filtration rate, and increased sodium and water reabsorption, and may progress into the hepatorenal syndrome, a functional and potentially reversible renal impairment in severely ill patients (22). Studies in non-azotaemic cirrhotic patients suggest that circulatory dysfunction with a decrease in cardiac output combined with splanchnic arterial vasodilatation and activation of the RAAS contribute to renal dysfunction and the hepatorenal syndrome (21, 24). Angiotensin II mainly acts on the efferent arteriole, and a low dose of an ACE inhibitor may induce a significant reduction in glomerular filtration and a further reduction in sodium excretion, even in the absence of a change in arterial blood pressure. This suggests that the integrity of the RAAS is important for the maintenance of renal function in cirrhotic patients and that RAAS overactivity does not solely contribute to the adverse renal vasoconstriction.

The circulation of the extremities

The cutaneous and muscular circulations may be increased in patients with cirrhosis (20). Palmar erythema, spider naevi and potatory face were early recognised as clinical signs of cutaneous hyperperfusion. These types of circulatory abnormalities illustrate capillary hyperperfusion and the presence of arteriovenous fistulae.

Abnormalities in the regulation of the circulation

Autonomic dysfunction

Cirrhosis is often associated with autonomic neuropathy which has become evident from studies of haemodynamic responses to standard cardiovascular reflex tests, such as heart rate variability and isometric exercise (2, 11, 25). Most studies on these issues have found a high prevalence of autonomic dysfunction in cirrhosis with associations with liver dysfunction and survival (13, 26). Most studies have focused on defects in the SNS, but the importance of vagal impairment for sodium and fluid retention has been shown (2, 25, 26). Sympathetic responses to exercise are clearly impaired (27, 28).

Similarly, blood pressure responses to orthostasis are impaired, probably because of a blunted baroreflex function in advanced cirrhosis (29). Abnormal cardiovascular responses to vasoconstrictors have been reported in patients with cirrhosis (20), and there is experimental evidence that haem oxygenase mediates hyporeactivity to phenylephrine in the mesenteric vessels of cirrhotic rats with ascites (30). Administration of captopril partly corrects the parasympathetic dysfunction in cirrhosis, which indicates that the vagal component is to a certain extent caused by neuromodulation with angiotensin II (26). Involvement of the RAAS is also supported by data that show normalisation of cardiac responses to postural changes after administration of canrenone, an aldosterone antagonist, to compensated cirrhotic patients (31). Interestingly, the vasoconstrictor hyporeactivity seems to be reversible by such antioxidants as vitamin C, which indicates that oxidative stress plays a role in vascular hyporeactivity and that antioxidant therapy could possibly have a role in these complications in cirrhosis (32). The pathophysiological basis underlying the autonomic dysfunction in cirrhosis is unknown, but relationships to the severity of the liver disease, mortality and reversibility after liver transplantation point to hepatic metabolism and increased NO production, and reduced vasoconstrictor sensitivity with postreceptor defects. This provides some explanation for the vascular hyporeactivity in cirrhosis.

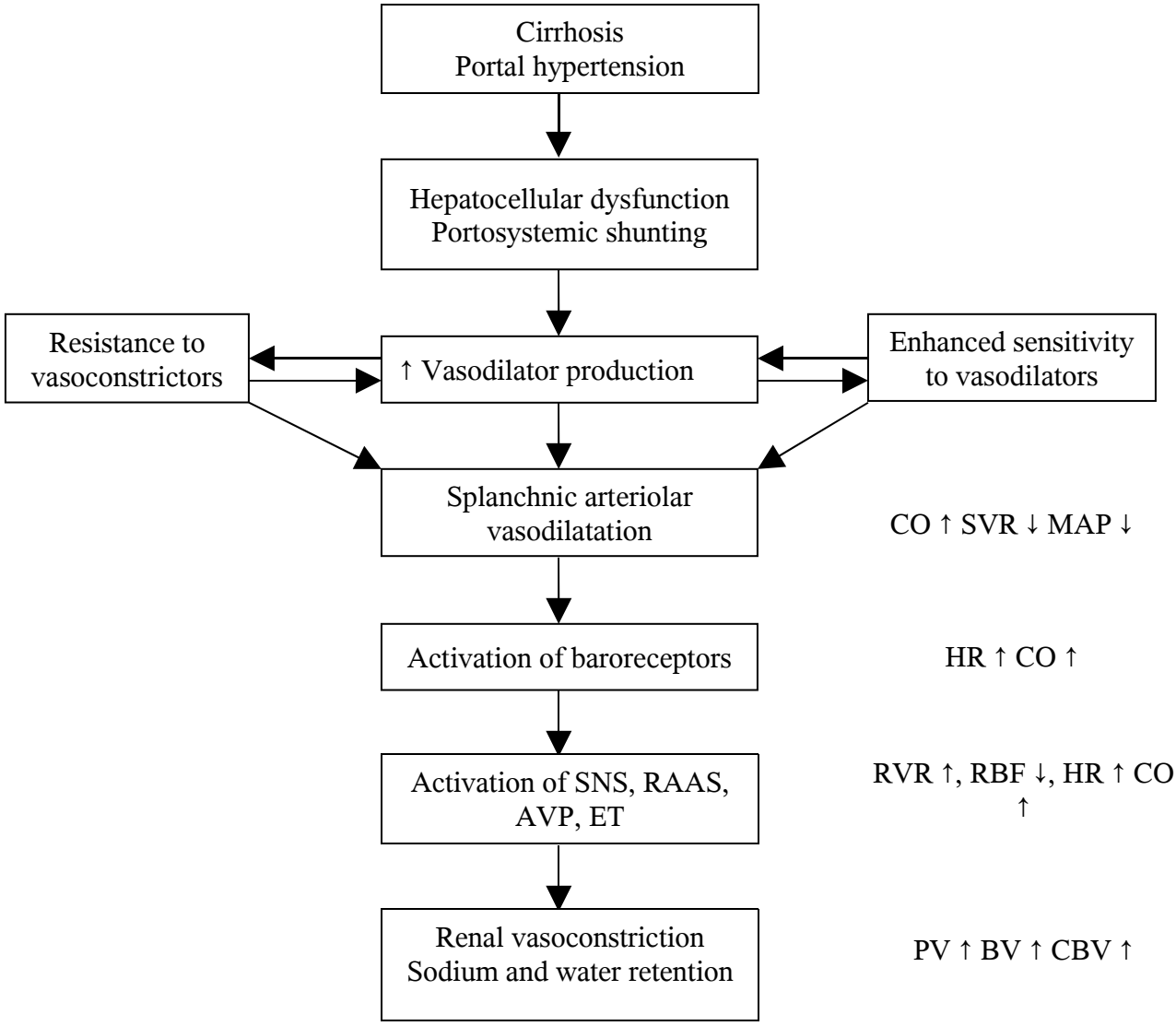

Figure 1: Schematic flow chart representing the hemodynamic changes in Cirrhosis.

Central blood volume (CBV), cardiac output (CO), heart rate (HR), plasma volume (PV), Blood volumes (BV), renal vascular resistance (RVR), renal blood flow (RBF), systemic Vascular resistance (SVR), Mean arterial pressure (MAP), SNS, sympathetic nervous system; RAAS, renin–angiotensin–aldosterone system; AVP, arginine vasopressin; ET, endothelin.

Arterial blood pressure and baroreceptor function in cirrhosis

The level of the arterial blood pressure, which depends on the cardiac output and the systemic vascular

resistance, is kept low normal in cirrhosis as a circulatory compromise between the vasodilatating and counter-regulatory vasoconstricting forces affecting both vascular resistance and arterial compliance. There is a relationship between the degree of arterial hypotension in cirrhosis and the severity of disease, signs of decompensation, and survival (19, 20). SNS, RAAS, vasopressin and ET-1 are all important vasoconstrictors involved in the maintenance of the arterial blood pressure in cirrhosis (20). Some of the potent vasodilators including, Nitric oxide (NO), endocannabinoids, endotoxins and andamide may contribute to the hyperkinetic state and arterial hypotension in cirrhosis (33, 34). The arterial blood pressure possesses a circadian variation. In cirrhosis, the arterial blood pressures are reduced during the day, whereas at night the values are normal, which indicates an abnormal blood pressure regulation (35). Whereas the baroreflex sensitivity (BRS) may be normal in early cirrhosis (36), there is substantial evidence that BRS is impaired in patients with advanced disease (37, 38). Together with a flat blood pressure/heart rate slope as found during 24 h ambulatory blood pressure monitoring, the low BRS contributes to the dysregulation of the arterial blood pressure, although the precise mechanism is unknown (35, 38).

Evaluation of the Autonomic function system

Failure or dysfunction of some portion of the ANS can affect various bodily functions, and a variety of symptoms may be attributable to autonomic dysfunction. Common symptoms of autonomic dysfunction are listed in the table. The autonomic symptoms recognized by clinicians most commonly are those which relate to failure of cardiovascular control (41).

Table 4: Common Symptoms of Autonomic Dysfunction

- Postural hypotension with symptoms (dizziness or fainting on standing)
- Gastric symptoms: gastric retention, nausea nd vomiting, postprandial epigastric bloating, early satiety
- Bowel disorders: nocturnal diarrhea, altered bowel habits, fecal incontinence

- Sweating disorders: Hypohidrosis, hyperhidrosis, gustatory diaphoresis
- Impotence (males)
- Dry eyes/mouth
- Failure of papillary accommodation (blurring of vision)
- Bladder dysfunction: urinary retention, impairment of bladder sensation

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Assessment of ANS function

Since many of the disorders of ANS function are manifested by effects on heart rate and blood pressure, several techniques have been devised for assessment of these functions.

Autonomic function testing laboratories have evolved over the years and equipments have been developed that allow for detailed and rather elaborate analysis techniques. The testing modalities available most widely are those that record heart rate and blood pressure. Testing protocols which allow for evaluation of both the sympathetic and parasympathetic arms of baroreflexes have been devised, and many are now relatively standardized (39, 40, 41).

Physiological tests that assess the SNS function

1) BP response to sustained handgrip

The blood pressure of the patient is taken three times before the manoeuvre with the help of a modified sphygmomanometer. The patient is asked to grip the inflatable rubber bag and apply maximum voluntary pressure possible. A reading from the attached mercury manometer is taken during maximum voluntary contraction. Thereafter, the patient is asked to maintain 30% of maximum voluntary contraction for as long as possible up to five minutes. Blood pressure is measured at one minute intervals during the handgrip. The result is expressed as the difference between the highest diastolic blood pressure during the handgrip exercise and the mean of the three diastolic blood pressure readings before the handgrip began.

Normal response is an increase in diastolic BP by ≥ 16 mm Hg. SNS dysfunction is said to be present, if the raise in diastolic BP is < 10 mm Hg.

2) BP response to standing

This test measures the subject's blood pressure with a sphygmomanometer while he was lying quietly and one minute after he is made to stand up. The postural fall in blood pressure is taken as the difference between the systolic pressure lying and the systolic blood pressure standing. The test is repeated three times and the mean is calculated.

An abnormal response is defined as a fall in systolic BP by atleast 20 mm Hg and in diastolic BP by at least 10 mm Hg.

3) BP response to immersion of hand in cold water

The patient is asked to immerse the hand in ice water (1-4ºC) for one minute. Blood pressure is recorded in the other arm at the end of one minute. Systolic and diastolic BP rise by 10-20 mm normally. Afferent for this pathway is the spinothalamic tract.

5) Sweat response

QSART: Quantitative Sudomotor Axon Reflex test is a measure of the regional autonomic function mediated by acetyl choline induced sweating. A reduced or absent response indicates a lesion of the post ganglionic sudomotor neuron.

TST: Thermoregulatory sweat test is a qualitative measure of regional sweat production in response to an elevation in body temperature. An indicator powder placed on the anterior body surface changes colour with sweat production during temperature elevation.

Combining QSART and TST results will determine the site of lesion. A postganglionic lesion is present if both QSART and TST show absent sweating. In a preganglionic lesion, QSART is intact, but TST shows anhidrosis.

Physiological tests that assess the PNS function

1) Heart rate response to long valsalva maneuver

The subject is seated quietly and then asked to blow into a mouthpiece attached to a manometer, holding it at a pressure of 40 mm Hg for 15 seconds while a continuous electrocardiogram (ECG) is recorded. The manoeuvre is repeated three times with one minute interval in between and results are expressed as:

Valsalva ratio = longest R-R interval after the manoeuvre ÷ shortest R-R interval during the manoeuvre.

The mean of the three Valsalva ratios is taken as the final value. Normal ratio is ≥ 1.21 and PNS dysfunction is said to be present if the ratio is < 1.10.

2) Heart rate variation during deep breathing

The subject is asked to breathe deeply at six breaths/min (five seconds “in” and five seconds “out”) for one minute. An ECG is recorded throughout the period of deep breathing and onset of each inspiration and expiration is marked on ECG paper. The maximum and minimum R-R intervals during each breathing cycle are measured with a ruler and converted to beats/min. The results of the test were expressed as the mean of the difference between maximum and minimum heart rates for the six measured cycles in beats/min.

Normal response is > 15 beats per minute. PNS dysfunction is present if the difference is < 10 beats per minute.

3) Immediate heart rate response to standing

The test is performed with the subject lying quietly on a couch while the heart rate is recorded continuously on an electrocardiograph. The patient was then asked to stand unaided and the point at starting to stand was marked on ECG paper. The shortest R-R interval at or around the 15th beat and the longest R-R interval at around the 30th beat after starting to stand are measured with a ruler. The characteristic heart rate response is expressed by 30:15 ratios.

Normal ratio is > 1.04 and PNS dysfunction is present, if the ratio is < 1.00.

Pharmacological tests

Pharmacological assessments can help localize an autonomic defect to the central or peripheral nervous system.

1) Measurement of plasma Norepinephrine (NE) level in supine position and 5 min after standing. Supine values are reduced in postganglionic disorders (eg: autonomic neuropathy) and may fail to raise on standing in pre or postganglionic disorders
2) Administration of Tyramine (releases NE from postganglionic terminals) and phenylephrine (denervation supersensitivity- directly acting α1- agonist) is often used to evaluate postganglionic adrenergic function.

Disadvantages of the standard tests

- Some of the tests are provocative that require a stimulus (cold pressor)
- Cumbersome to perform and interpret (eg: valsalva ratio)
- Difficult to reproduce

Advantage of the standard tests

- Can help localize the site of the autonomic neuropathy (whether peripheral or central)

The latest development in the assessment of ANS function includes the analysis of heart rate variability.

HEART RATE VARIABILITY

Introduction

The heart rate and rhythm are largely under the control of autonomic nervous system. **Heart**

rate variability (HRV) is the oscillations in the interval between consecutive heart beats as well as oscillations between consecutive instantaneous heart rate, ie, it describes the variations of both instantaneous heart rate and RR intervals (from the electrocardiogram tracing). Other terms have been used in the literature include, **cycle length variability, heart period variability, RR variability**, and **RR interval tachogram**, and they, more appropriately emphasize the fact that it is the interval between consecutive beats that is being analyzed rather than the heart rate per se (12).

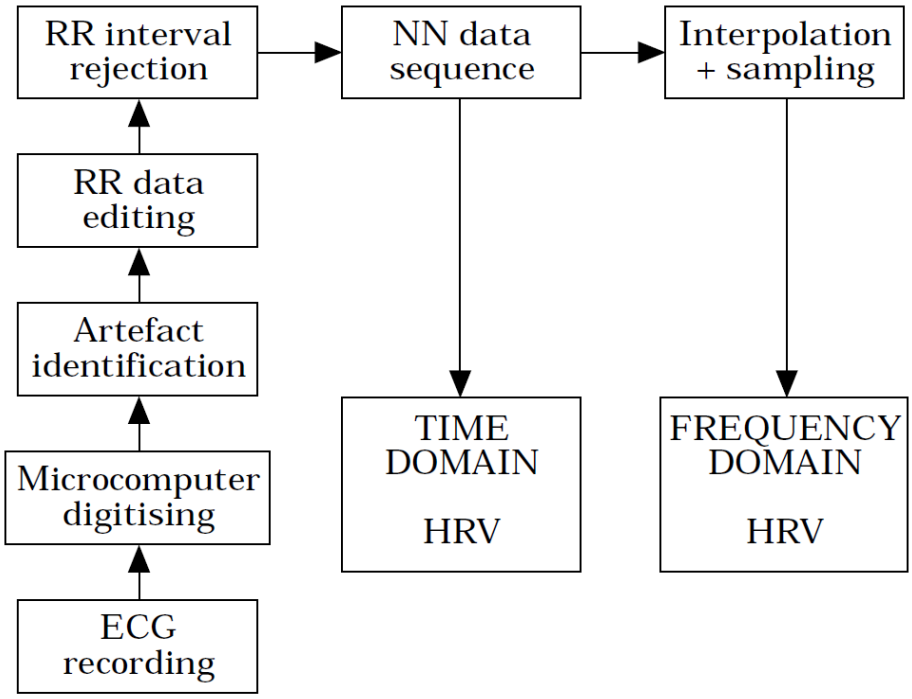
Back ground

The clinical use of HRV was well recognized, when Hon and Lee, in 1965 (42), showed that, fetal distress was preceded by alterations in HRV before any notable change occurred in the heart rate itself. During 1970's , Ewing et al (39) made simple bedside test of short term RR difference to detect a neuropathy in diabetic patients. The association of higher post-infarction mortality with reduced HRV was later shown by Wolf et al (43). In 1981 Akselrod et al (44) introduced first power spectral analysis of heart rate fluctuations to quantitative evaluation.

The clinical importance of HRV became apparent in the late 1980s when it was confirmed that HRV was a strong and independent predictor of mortality following an acute myocardial infarction (45). With the availability of new, digital, high frequency, 24-h multi-channel electrocardiographic recorders, HRV has the potential to provide additional valuable insight into physiological and pathological conditions and to enhance risk stratification.

The European society of Cardiology and North American society of pacing and electrophysiology have contributed a Task force to develop appropriate standards to measure HRV, which has been followed in this study (12).

Figure 2: Flow chart summarizing individual steps used when recording and processing the ECG signal in order to obtain data for HRV analysis



Measurement of heart rate variability

Time domain methods

Variations in heart rate may be evaluated by a number of methods. Perhaps the simplest to perform are the time domain measures. With these methods either the heart rate at any point in time or the intervals between successive normal complexes are determined. In a continuous electrocardiographic (ECG) record, each QRS complex is detected, and the so-called normal-to-normal (NN) intervals (that is all intervals between adjacent QRS complexes resulting from sinus node depolarizations), or the instantaneous heart rate is determined. Simple time-domain variables that can be calculated include the

mean NN interval, the mean heart rate, the difference between the longest and shortest NN interval, the difference between night and day heart rate, etc.

Other time domain measurements that can be used are variations in instantaneous heart rate secondary to respiration, tilt, Valsalva manoeuvre, or secondary to phenylephrine infusion. These differences can be described as either differences in heart rate or cycle length. The important time domain measures are:

- Mean R-R (msec): Mean of the normal-to-normal (N-N) interbeat interval.
- SDNN: Standard deviation (SD) of all the R-R intervals in a given period, expressed in milliseconds.
- SDANN (msec): Standard deviation of the average of N-N intervals for each 5-min period over 24 hr. SDANN is mainly accepted to represent the sympathetic component of autonomic function.
- r-MSSD (msec): Root mean square successive differences (the square root of the mean of the sum of the squares of differences between adjacent N-N intervals).
- pNN50 (%): Percentage of adjacent N-N intervals that are >50 msec apart (pNN50). NN50 is the number of interval differences of successive NN intervals >50 msec and pNN50 is the proportion derived by dividing the NN50 by the total number of NN intervals. pNN50 and r-MSSD are mainly accepted to represent the parasympathetic component of autonomic function.

Frequency domain methods

The second method of assessing HRV is by the use of power spectral density analysis or frequency domain analysis. PSD analysis provides the basic information of how power (ie variance) distributes as a function of frequency. Power spectral analysis plots the distribution (spectra) of HR oscillations in the frequency domain by mathematically transforming sequential R-R intervals on the electrocardiogram into specific frequency components.

Methods for the calculation of PSD may be generally classified as non-parametric and parametric. In most instances, both methods provide comparable results. The advantages of the non-parametric methods are: (a) the simplicity of the algorithm employed (Fast Fourier Transform -FFT- in most of the cases) and (b) the high processing speed.

Fast Fourier transform algorithm can plot the relative energy of different frequency components of HRV. The Fourier transform is based on the Fourier theorem, which states that any periodic signal can be expressed as a sum of an infinite set of sine and cosine functions with different characteristic periods of oscillation and different weighting coefficients. The Fourier transform projects the complex periodic oscillation in R-R interval onto each of these periodic basis functions in much the same way that vectors in 3 dimensional space are projected onto the 3 basis vectors that define our visual space. The relative power of each point in the frequency domain subsequently can be obtained.

Spectral components

The three main components that are distinguished in a spectral calculation from short term ECG recording are (44)

VLF - Very low frequency

LF - Low frequency

HF - High frequency

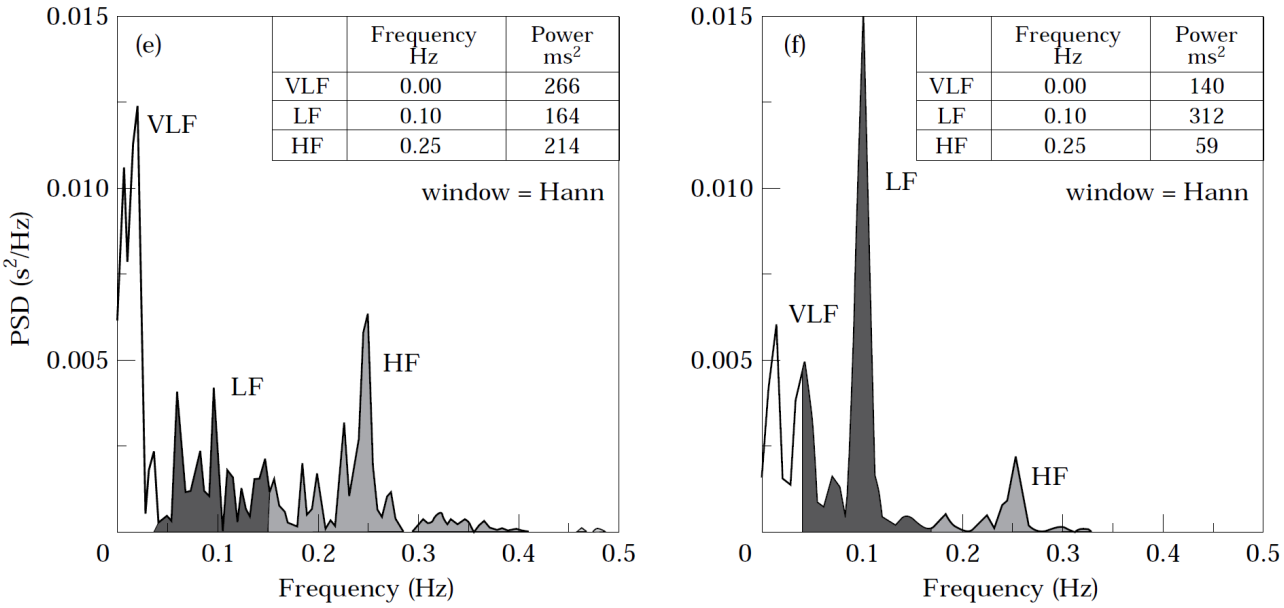
The distribution of power and central frequency of LF and HF are not fixed but may vary in relation to changes in autonomic modulations of the heart period (46). The physiological explanation for VLF component is much less defined and the existence of a specific physiological process attributable to these heart period changes might even be questioned. Thus VLF assessed from short-term recordings (e.g. 5 min) is a dubious measure and should be avoided when interpreting the Power spectral density (PSD) of short-term ECGs.

The measurement of VLF, LF and HF power are usually made in absolute values of power (millisecond squared). Their frequency range is given as follows

	Range (Hz)
VLF	0.0 - 0.04
LF	0.04 - 0.15
HF	0.15 - 0.4

LF and HF are also measured in normalized units (47), which represent the relative value of each power component in proportion to the total power minus the VLF component. So the representation of LF and HF in normalized units emphasizes the controlled and balanced behavior of the two branches of ANS. Moreover, normalization tends to minimize the effect on the values of LF and HF components of the changes in total power. Nevertheless, n.u should be always quoted with absolute values of LF and HF power in order to describe in total the distribution of power in spectral components. In this study, we have quoted absolute value for LF and HF and also the normalized units for LF and HF. The ratio of LF to HF gives the autonomic modulation. If the modulations are constant, the interpretations of the results of frequency analysis are well defined. In our physiological mechanism the heart period modulations are taking place every movement and so these changes are seen in LF and HF components.

Figure 3: HRV analysis with the Power Spectral Density (PSD) of a healthy person in supine (left) and upright (right) posture, showing the VLF, LF and HF components. LF: dark shaded area; HF: light shaded area, VLF: white area



Thus spectral analysis are taken on the entire 24-hrs period as well as spectral results are obtained from shorter segments (5 min) averaged over entire 24hrs also (48). In the present study, we have used short term (5 min) HRV analysis. It should be remembered that the components of HRV provide measurement of the degree of autonomic modulation rather than of the level of autonomic tone.

Physiological correlations of HRV

An understanding of the modulatory effects of neural mechanisms on the sinus node has been enhanced by spectral analysis of HRV (47). The efferent vagal activity is a major contributor to the HF component, as seen in clinical and experimental observations of autonomic maneuvers such as electrical vagal stimulation, muscarinic receptor blockade, and vagotomy. More controversial is the interpretation the LF component which is considered by some (49, 50, 51) as a marker of sympathetic modulation (especially when expressed in normalized units) and by others (52) as a parameter that includes both sympathetic and vagal influence. This discrepancy is due to the fact that in some conditions associated with sympathetic excitation, a decrease in the absolute power of the LF component is observed. It is important to recall that during sympathetic activation the resulting

tachycardia is usually accompanied by a marked reduction in total power, whereas the reverse occurs during vagal activation. When the spectral components are expressed in absolute units (milliseconds squared), the changes in total power, influence LF and HF in the same direction and prevent the appreciation of the fractional distribution of the energy. This concept is exemplified in showing the spectral analysis of HRV in a normal subject during control supine condition and 90^0 head-up tilt(Montano et al 1994). Because of the reduction in total power, LF appears as unchanged if considered in absolute units. However, after normalization an increase in LF becomes evident. Similar results apply to the LF/HF ratio also.

LF and HF can increase under different conditions. An increased LF (expressed in normalized units) is observed during 90^0 tilt, standing, mental stress, and moderate exercise in healthy subjects, and during moderate hypotension, physical activity, and occlusion of a coronary artery or common carotid arteries in conscious dogs. Conversely, an increase in HF is induced by controlled respiration, cold stimulation of the face, and rotational stimuli. To conclude HRV analysis has got wide application in clinical medicine such as diabetic neuropathy, Post Myocardial infarction (sudden death, malignant arrhythmias), autonomic function disturbances, myocardial dysfunctions, CVS drug therapy, vagal dominance identification, exercise training and many other clinical disorders.

Advantages of HRV analysis over standard autonomic function tests

- Single test gives information on both the sympathetic and the parasympathetic nervous system function
- Easy to perform and to interpret for the clinician
- Patient friendly (no need for provocative stimuli)
- Easily reproducible

Disadvantage of HRV analysis over standard autonomic function tests

- Can not localize the site of autonomic neuropathy (whether central or peripheral)

Definitions and Interpretations

- Mean RR interval: Mean of all the R-R intervals
- Parasympathetic function
  - SDNN : SD of N to N (R-R) intervals
  - HF power (ms^2;150-400 mHz)
  - HF (normalized units): HF (power) ÷ [LF + HF (power)]
- Sympathetic and parasympathetic function: LF power (ms^2; 4-150mHz): Indicates sympathetic more than parasympathetic function
- Sympathetic function: LF (normalized units): LF (power) ÷ [LF + HF (power)]

Effects of ANS dysfunction on the natural history of cirrhosis

The prevalence autonomic dysfunction (AD) in cirrhosis has varied from 8% - 80% in different series (9, 53-57), depending on the underlying severity of liver disease.

ANS dysfunction and the etiology of liver disease

Autonomic nervous dysfunction is a known complication of diabetes (39) and alcohol abuse (58). Autonomic damage is expected in some patients with alcohol related cirrhosis since autonomic neuropathy, especially of vagal origin, is seen in chronic alcoholics (58). However, it is well established from studies that AD occurs irrespective of the etiology of liver disease: ethanol or non-ethanol related (10, 40, 55).

ANS dysfunction and severity of liver disease

Hendrickse et al in 1993 (59), described that chronic liver disease is accompanied by a number of circulatory changes including impairment of cardiovascular autonomic reflexes. This occurs

irrespective of the aetiology of liver disease, increases in prevalence and severity with worsening hepatic function, and is related at least in part to an autonomic neuropathy. Parasympathetic abnormalities predominate and, although largely subclinical, they may play a role in the altered fluid homeostasis and neurohumoral disturbances associated with cirrhosis. On prospective follow up, the presence of autonomic impairment was associated with a five-fold increased mortality, largely from sepsis and variceal haemorrhage. Defective responses to such stressful events as a result of an afferent defect could possibly explain these findings.

Fleckenstein et al (10) showed that the prevalence of AD was 14.3% in Child's A, 31.3% in Child's B, and 60%Child's C patients. Six patients died during a median observation period of 10months, and all had AD. On the basis of this observation, they suggested that consideration should be given for early liver transplantation in patients with advance liver disease and autonomic neuropathy

Fleisher et al (60), in a longitudinal study to determine whether the severity of liver disease correlated with measures of heart rate variability, studied 21 patients being evaluated for liver transplantation. Heart rate variability was determined for a series of 500 consecutive R-R intervals during quiet breathing. Standard deviation, pNN50, a marker of parasympathetic function, and approximate entropy (ApEn), a recently described measure of regularity, were calculated. Four standard tests of autonomic function were also performed. pNN50 was significantly reduced in all liver disease patients compared to controls ($P < 0.05$). Both standard deviation and ApEn were significantly reduced in Child's class C patients suggesting a generalized dysfunction in cardiovascular homeostasis. ApEn was significantly lower in the nonsurvivors during follow-up than the survivors ($P < 0.05$). The authors concluded that, increasing severity of liver failure is associated with a reduction in total heart rate variability and regularity & measurement of heart rate variability offers a simple, noninvasive means of assessing the cardiovascular and autonomic effects of liver disease, particularly in those awaiting liver transplantation.

Coelho et al (61), evaluated autonomic function in 22 cirrhotic patients (55% alcoholic cirrhosis) by using the 24 hour Heart Rate Variability study. The cirrhotic patients showed severe decrease in Heart Rate Variability when compared to healthy volunteers. The spectral analysis revealed marked decrease of average total power, with reduction of all components

(VLF, LF, HF), in the absence of significant difference in LF/HF ratio. Ascites had relationship with more pronounced autonomic impairment. On the other hand, alcohol related etiology did not influence Heart Rate Variability parameters. The authors, found significant positive correlations between SDNN (dependent variable) and Prothrombin activity ($r = 0.64$; $p = 0.001$), as well as with Serum Albumin ($r = 0.40$; $p = 0.05$), but not with Total Bilirubin ($r = -0.14$; $p = 0.51$). Prothrombin activity was the only independent predictor of autonomic dysfunction. The authors concluded that the greater the hepatopathy severity, the greater the heart rate variability impairment. Hepatocellular dysfunction indicators have more accuracy to demonstrate autonomic disturbances than cholestasis indicators.

Iga et al (62), evaluated abnormalities of autonomic nervous function in 50 patients with liver cirrhosis by 123I-metaiodobenzylguanidine (MIBG) myocardial scintigraphy and heart-rate variability. Echocardiogram, urinary nitrite and nitrate, and cathecholamines were examined. Washout rate of MIBG, LF/HF, and blood levels of norepinephrine increased and HF power decreased with the progression of Liver Cirrhosis. However, the urinary secretion of nitrite and nitrate were significantly increased only in cirrhotic patients with Child C. The authors concluded that autonomic abnormalities appear early in cirrhotics, and that these abnormalities can be detected by MIBG myocardial scintigraphy and analysis of heart-rate variability.

Ates et al (12), analyzed HRV using 24-hr ECG recording in 30 cirrhotic patients and 28 normal controls. The changes in HRV parameters involving the time domain measures were analyzed. The time-domain measures of HRV in cirrhotic patients were significantly reduced compared with those in the control group (for all parameters; $P < 0.001$). The severity of disease was associated with reduced HRV measures (for all parameters; $P < 0.001$). After the 2-year follow-up periods, HRV measurements in cirrhotic patients were significantly much lower in nonsurvivors than in survivors ($P<0.001$ for all). The authors concluded, that increasing severity of cirrhosis is associated with a reduction in HRV, which may be an indicator of poor prognosis and mortality for cirrhosis.

Thus, various studies have shown that AD does occur in cirrhosis and has a positive correlation with increasing severity of the underlying liver disease. With increasing liver disease, there is an over

activity of the sympathetic tone accompanied with a decrease in the parasympathetic tone. Also, there is reduced heart rate variability with increasing severity of cirrhosis liver.

ANS dysfunction and cholelithiasis

Chawla et al. studied the contribution of autonomic neuropathy to the formation of gallstones or gallbladder disease (due to impaired gallbladder emptying) in 123 patients (Child classes: A, 40; B, 45; C, 35) with cirrhosis liver. In all, 54 patients had gallstones and an additional 22 patients had other gallbladder disease (cholecystitis, common bile duct stones, or debris). Autonomic neuropathy was seen in 97 patients (one abnormal test in 48 and two or more in 49). The prevalence of gallstones was similar in Child A (57%), Child B (64%), and Child C (63%) cirrhosis. The gallstones or gallbladder disease was not increased in women, blacks, diabetics, or alcoholic cirrhotics. The prevalence of gallbladder disease was increased in patients with autonomic neuropathy (51% vs 35%, $P = 0.08$); in patients with Child C cirrhosis, gallstones ($P = 0.018$) and gallbladder disease ($P = 0.03$) were seen more commonly in patients with autonomic neuropathy. Thus, the authors concluded that autonomic neuropathy may contribute to the formation of gallstones in patients with advanced cirrhosis, perhaps by impairing gallbladder and sphincter of Oddi dysmotility.

ANS dysfunction and variceal bleed.

Varghese at al (11), studied the influence of autonomic dysfunction on the variceal bleed (using standard ANS function tests) in fifty cirrhotics (variceal bleeders: 34) belonging to either sex and Childs B class. True AD was noted in 10 patients i.e. 20%. Parasympathetic dysfunction alone was positive in 19 (38%) and sympathetic in 10 patients (20%). The E:I ratio ($p < 0.001$) and an increase in diastolic B.P. during sustained hand grip ($p < 0.04$) were significantly positive amongst variceal bleeders. They concluded that ‘True’ autonomic dysfunction can predispose a cirrhotic to variceal bleed. It was postulated that, in presence of AD, a variceal bleed may fail to elicit the compensatory cardiovascular reflex response and may cause an inappropriate pooling of blood in the splanchnic

circulation, thereby increasing the risk of further variceal bleed.

Effect of postural variation on cardiovascular autonomic responses:

Laffi et al (37), performed a study on the effects of passive head tilting on the cardiovascular autonomic responses, in 15 cirrhotic patients with ascites and in 13 healthy subjects, using heart rate variability analysis and measurement of plasma norepinephrine levels. In the supine position, no significant differences in the Power Spectral Analysis data (LF, HF) were observed between the control subjects and cirrhotic patients, who had higher plasma norepinephrine levels. In healthy subjects, tilting was associated with an increase in the LF and arterial pressure and a decrease in the HF. In contrast, patients with cirrhosis showed a decrease of both LF and HF. Consequently, the LF/HF ratio significantly increased in healthy subjects, whereas it was unchanged in cirrhotic patients. The LF component of the diastolic pressure also decreased during tilting in cirrhotic patients. Plasma norepinephrine increased after tilting in both groups. They concluded that the autonomic responnse to passive tilting is impaired in cirrhotic patients with ascites at both the cardiac and vascular levels, as a result of an altered sympatho-vagal balance, with reduced sympathetic predominance. They also postulated that since these alterations occurred despite an appropriate response to the tilting of plasma norepinephrine, it pointed to a receptorial or postreceptorial site of the autonomic impairment.

More recently Moller et al (64), studied the humoral and central hemodynamic responses to changes with posture in 23 patients with cirrhosis (Child-Pugh class B/C: 21) and 14 healthy controls in the supine position and after 60° passive head-up tilting for a maximum of 20 minutes. After the head-up tilting, the central blood volume (CBV) decreased in both groups, but the decrease was significantly smaller in patients than in controls. The absolute increases in circulating norepinephrine and renin after head-up tilting were significantly higher in the patients than in the controls. They concluded that head-up tilting decreases the central blood volume less in patients with cirrhosis, and it might be because of a differential regulation of central hemodynamics in patients with cirrhosis, with a higher activity of the

RAAS.

ANS function after liver tansplantation

Though, autonomic dysfunction is a well recognized complication of end-stage liver disease (ESLD), but there is little information on how liver transplantation (LT) affects this problem. Carey et al (65), prospectively evaluated autonomic function in patients with ESLD before and after LT. Autonomic reflex screen (ARS) was performed on 30 patients with ESLD prior to transplantation. A 10-point composite autonomic score (CAS) was calculated from these data. ARS was repeated after LT, and these scores were compared with the pre-LT ARS. Thirty patients (25 male, 5 female) with cirrhosis that were listed for LT were enrolled in the study and underwent ARS prior to LT. The average age was 55.4 ± 9.1 years. Indications for LT included hepatitis C virus (14), cryptogenic cirrhosis (5), alcoholic cirrhosis (4), and other (7). The mean native Model for End-Stage Liver Disease (MELD) score at ARS was 17.0 ± 5.0. Prior to LT, 86.7% of patients had evidence of autonomic dysfunction. Mean CAS was 2.7 ± 2.2. Sudomotor function was disturbed in 66%, parasympathetic function was disturbed in 57%, and adrenergic function was disturbed in 37%. There was no relationship between pre-LT CAS and age, gender, diabetes, etiology of liver disease, or MELD score. Twenty-one patients (17 male, 4 female) had repeat ARS a mean of 9 ± 6.2 months after LT. The mean native MELD score at the time of ARS testing was 18.1 ± 4.3. Mean pre-LT CAS in this group was 3.0 ± 2.4. Pretransplant CAS was not related to age, gender, diabetes, or MELD score. Autonomic dysfunction improved after LT (CAS pre-LT, 3.0, versus CAS post-LT, 1.9, $P = 0.02$). There was no relationship between post-LT CAS and age, gender, diabetes, etiology of liver disease, immunosuppression, or type of transplant. The authors concluded that, autonomic dysfunction is common in patients with ESLD, with over 86% having abnormal testing. Sixty-three percent of patients with cirrhosis with autonomic dysfunction show improvement after LT.

Materials and Methods

Patients with a diagnosis of cirrhosis of the liver were included in the study group. The diagnosis of cirrhosis was made by clinical, biochemical and ultrasound criteria. The patients were grouped according to the modified Child-Pugh classification and also according to their bleed status (Variceal bleeders or non-bleeders)

Exclusion criteria were

- Use of Alcohol or tobacco products
- Diabetes, hypertension, coronary artery disease, rhythm or conduction disturbances
- Severe anemia (Hb < 7 gm/dl), electrolyte disorders
- Recent bleed (< 4 wk), thyroid disease, renal or cardiac failure
- Known neurological disorder
- Hepatocellular carcinoma

Study period was from June 2007 to December 2008. HRV analysis was performed in the subjects between June and December 2007 and the patients were further followed up for a period of one year during outpatient visits or by telephonic calls at frequent intervals or till death. The cause of death was ascertained in each patient.

Controls were age and sex matched healthy adults. The control group consisted of consorts or relatives of the patients.

Ethical committee approval was obtained prior to the commencement of the study.

Study protocol

The tests were carried out in the Electrophysiology lab, Department of Physiology, Stanley Medical College, Chennai.

All the recordings were done between 10.00 am and 1.00 pm. The laboratory environment was quiet with an ambient temperature between 25 - 28 degrees Celsius and the lighting subdued. Subjects were asked to empty their bladder before the tests. The tests did not involve intravascular instrumentation or administration of any drugs at any stage. Patients on Beta blockers were asked to withhold the drug for 1 week prior to testing. Diuretics were stopped on the day of the testing. No patient underwent paracentesis in the three weeks preceding the study.

Equipment

ECG was acquired using RMS Polyrite D hardware 2.2 (India), and instantaneous heart rate & RR intervals were continuously plotted using RMS 2.2 software on a Microsoft Window-based PC (The RMS polyrite software 2.2 helps to save multiple records and is provided with additional filter settings, calculation tools, automated analysis and auto report generation) (Figures 4 & 5). Respiratory movements were recorded using a respiratory belt which detected both inspiration and expiration.

Measurement of HRV

The subjects were made to sit in the lab for 10min to get accustomed to the new environment after emptying the urinary bladder, with a prior instruction of not to have anything by mouth for 1½ hours before test. They were also instructed not to have tea or coffee on the morning of the test. After obtaining an informed consent and explaining about the procedure, the test was carried out as follows -

1) Electrodes were placed in the left and right shoulder and in the right leg.

2) Respiratory belt was tied around the chest at the level of nipple to record respiratory movement.

3) The electrodes and the respiratory belt were connected to RMS polyrite D equipment.

4) ECG was recorded for 10 minutes to determine the HRV at supine rest with the eyes closed with normal quite respiratory movement (12-16/min).

5) After recording in the supine position the subjects were asked to stand without support on a wooden

plank and the recordings were repeated.

Technique of Heart Rate Variability Analysis:

The ECG recording was checked for artifacts and ectopics. Recordings which showed such conduction disturbances were excluded. A 5-minute interval in the recording for selected for analysis of the data in the time domain (Mean R-R interval & Standard deviation of N-N intervals) and frequency domain [Low Frequency (LF) & High frequency (HF) {in power and normalized units (n.u)} and LF/HF ratio] analysis. (Fig 6)

All the derivations complied with the standards recommended by the Task force of the European Society of Cardiology and the North American Society for Pacing and Electrophysiology (12).

Statistical analysis

Data were expressed as Mean ± standard deviation. Statistical analyses were carried out using Independent sample *t* test or one-way ANOVA test, where appropriate. P value < 0.05 was considered as significant.

Observation and Results

51 patients were enrolled in the study. 13 patients were later excluded (Not co-operative for HRV analysis: 3; Alcohol consumption: 5; Ectopic beats/Movement artifacts: 3).

38 cirrhotic men with an age range between 20-62 years were finally included for analysis.

The etiology of cirrhosis was Hepatitis B: 15; Hepatitis C: 11; Wilson disease: 2 and Cryptogenic cirrhosis: 10. (Table 5)

Table 5: Characteristics of the study population

Variable	Number
No. of patients	38
Variceal bleed	19
Etiology	
Hepatitis B	15
Hepatitis C	11
Wilson disease	2
Cryptogenic	10
Child Class A/B/C	6/19/13
Survivors	26
Cause of death	
Variceal bleed	5
Spontaneous Bacterial Peritonitis	4
Hepatoma	2
Leptospirosis with renal failure	1

6 patients belonged to Child-Pugh (CP) class A, 9 to CP class B and 13 to CP class C. 18 patients (belonging to CP class B & C) had ascites requiring diuretics for control. 19 patients each were variceal bleeders and non-bleeders.

All patients were followed up till the end of the study period. 12 patients passed away on follow up. The causes of death were- Variceal bleed: 5 patients; Spontaneous Bacterial Peritonitis: 4 patients; Hepatoma: 2 patients and Leptospirosis with renal failure: 1 patient. (Table 5). Symptoms of autonomic dysfunction (postural dizziness) were present in 6 patients (Child Class B: 3; Class C: 3)

HRV analysis

Cases vs Controls

Table 6: Cases vs Controls (Supine position)

	Cases (38)	Controls (22)	P value
Mean RR	0.82± 0.14	0.88 ±0.13	0.088
SDNN	22.42± 13.6	42.31 ±19.07	**0.000**
LF (power)	19.0 ±15.6	14.06 ±9.05	0.181
HF (power)	12.64 ±15.69	38.4 ±15.95	0.853
LF (nu)	67.86 ±16.38	57.91 ±17.25	**0.03**
HF (nu)	33.5 ± 17.82	41.97 ±17.24	0.62
LF/HF	2.25 ±3.07	1.91 ±1.56	0.627

Cirrhotics have a significantly lower SDNN (implying reduced HRV and a decreased parasympathetic tone); higher LF (n.u) & LF/HF ratio in supine (Increased sympathetic tone) posture.

Table 7: Cases vs Controls (Upright position)

	Cases (38)	Control (22)	P value
Mean RR	0.77 ±0.15	0.71± 0.1	0.062
SDNN	20.23 ±14.08	31.15± 10.92	**0.003**
LF (power)	17.19 ±13.6	28.47 ±15.44	**0.005**
HF (power)	10.85 ±11.25	8.5 ±5.91	0.368
LF (nu)	66.34 ±17.39	76.37 ±13.52	0.058
HF (nu)	32.28 ±16.2	21.13 ± 9.39	0.355
LF/HF	1.6 ±1.12	4.48 ±2.62	**0.000**

Cirrhotics have a significantly lower LF (power) in upright position ie, lowered sympathetic tone compared to controls in upright posture. Also, they have a lower SDNN (implying reduced HRV) compared to controls in the upright position.

Table 8: Controls (Supine vs Upright posture)

Control (22)	Supine	Upright	P value
Mean RR	0.88± 0.13	0.71± 0.10	**0.00**
SDNN	42.31± 19.70	31.15± 10.92	**0.022**
LF (power)	14.06 ±9.05	28.47 ±15.44	**0.000**
HF (power)	13.43 ±15.95	8.50 ±5.91	0.182
LF (nu)	57.9 ±17.25	76.37 ±13.52	**0.001**
HF (nu)	41.97 ±17.24	21.13 ± 9.39	**0.001**
LF/HF	1.91 ±1.56	4.48 ±2.62	**0.000**

As the control subject assumes an upright posture from the supine position, an increase LF (power and

n.u) & LF/HF ratio with a decrease in SDNN, Mean R-R interval & HF (n.u) is observed. These changes imply the physiological compensatory increase in sympathetic tone associated with a decrease in parasympathetic tone as one assumes an erect posture.

Table 9: Cases (Supine vs Upright posture)

Cases (38)	Supine	Standing	P value
Mean RR	0.81 ± 0.14	0.77 ± 0.13	0.15
SDNN	22.42 ± 13.6	20.22 ± 14.08	0.49
LF (power)	19.0 ± 15.6	17.19 ± 13.6	0.59
HF (power)	12.64 ± 15.69	10.85 ± 11.25	0.57
LF (nu)	67.86 ± 16.38	66.34 ± 17.39	0.70
HF (nu)	33.5 ± 17.82	32.28 ± 16.2	0.32
LF/HF	2.25 ± 3.07	1.6 ± 1.12	0.22

On changing from the supine to the upright posture, the decrease in SDNN accompanied with an increase in LF power, observed in controls, was not seen in cirrhotics.

Child-Pugh Class A vs B vs C

Table 10: Child-Pugh Class A vs B vs C (Supine posture)

	CP class A (6)	CP class B (9)	CP class C (13)	P Value
Mean RR	0.83 ± 0.15	0.83 ± 0.15	0.79 ± 0. 13	0.65
SDNN	30.90 ± 16.08	21.16 ± 11.32	20.34 ± 15.08	0.25
LF (power)	38.63 ± 16.60	30.93 ± 13.68	29.97 ± 10.74	0.35
HF (power)	18.82 ± 10.93	14.99 ± 18.60	6.36 ± 10.93	0.18

LF (nu)	50.78 ± 20.84	69.03 ± 14.05	72.6 ± 17.65	**0.05**
HF (nu)	32.35 ± 16.26	35.85 ± 19.28	30.6 ±.17.12	0.596
LF/HF	2.48 ± 1.90	2.21 ± 3.34	2.2 ±.3.28	0.982

In the supine posture, there was an increase in LF (n.u), with increasing severity of cirrhosis i.e increased sympathetic tone with advanced liver disease (CP class A vs B/C)

Table 11: Child-Pugh Class A vs B vs C (Upright posture)

	CP class A (6)	CP class B (9)	CP class C (13)	P Value
Mean RR	0.75 ± 0.12	0.8 ± 0.13	0.74 ± 0.13	0.42
SDNN	20.42 ± 8.39	21.31 ± 15.91	18.56 ± 14.05	0.87
LF (power)	20.42 ± 6.48	19.33 ± 17.05	12.58 ± 9.01	0.32
HF (power)	16.2 ± 6.52	11.08 ± 12.4	8.05 ± 10.9	0.35
LF (nu)	67.65 ±16.26	66.78 ± 16.70	69.54 ±17.16	0.90
HF (nu)	39.6 ± 20.31	30.82 ± 14.09	31.02 ± 17.54	0.49
LF/HF	1.17 ± 0.41	1.92 ± 1.26	1.32 ± 1.04	0.20

No significant difference in the HRV parameters with increasing severity of cirrhosis (CP class A vs B vs C) in the upright posture (both SNS & PNS function)

Survivors vs non-survivors

Table 12: Survivors vs non-survivors (Supine position)

	Survivors (26)	Non Survivors (12)	P value
Mean RR	0.83 ± 0.15	0.79 ± 0.12	0.524
SDNN	21.98 ± 14.42	23.35 ± 12.18	0.777
LF (power)	17.30 ±14.22	22.68 ± 18.36	0.329
HF (power)	9.43 ± 11.25	19.61 ± 21.52	0.145

LF (nu)	69.99 ± 14.49	63.25 ± 19.79	0.244
HF (nu)	29.91 ± 14.52	35.85 ± 22.20	0.300
LF/HF	2.26 ± 2.91	2.23 ± 3.53	0.978

No significant difference in the HRV parameters (both sympathetic and parasympathetic function) was observed between and survivors and non-survivors in the supine position.

Table 13: Survivors vs non-survivors (Upright position)

	Survivors (26)	Non Survivors (12)	P value
Mean RR	0.781 ± 0.13	0.746 ± 0.129	0.45
SDNN	20.34 ± 15.41	18.90 ± 11.15	0.70
LF (power)	16.89 ± 14.73	17.85 ± 11.31	0.84
HF (power)	10.06 ± 10.24	12.56 ± 13.52	0.53
LF (nu)	67.88 ± 15.92	63.01 ± 20.58	0.43
HF (nu)	32.4 ± 15.83	32.02 ± 17.69	0.95
LF/HF	1.67 ± 1.22	1.43 ± 0.89	0.55

No significant difference in the HRV parameters (both sympathetic and parasympathetic function) was observed between and survivors and non-survivors in the upright position.

Bleeders vs Non-bleeders

Table 14: Bleeders vs Non-bleeders (Supine position)

Supine	Non-Bleeders (19)	Bleeders (19)	P value
Mean RR	0.80 ± 0.16	0.83 ± 0.121	0.488
SDNN	23.25 ± 14.45	21.58 ± 13.04	0.711
LF (power)	22.17 ± 14.15	15.83 ± 16.69	0.214
HF (power)	12.21 ± 13.86	13.08 ± 17.70	0.866
LF (nu)	69.18 ± 15.97	66.54 ± 17.12	0.626
HF (nu)	30.7947 ± 15.99	36.21 ± 19.28	0.300
LF/HF	3.07 ± 3.99	1.43 ± 1.44	0.102

No significant difference in the HRV parameters (both sympathetic and parasympathetic function) was observed between bleeders and non-bleeders in the supine postion.

Table 15: Bleeders vs Non-bleeders (Upright position)

Standing	Non-Bleeders (19)	Bleeders (19)	P value
Mean RR	0.76 ± 0.15	0.78 ± 0.11	0.60
SDNN	21.73 ± 16.72	18.9 ± 11.8	0.55
LF (power)	18.41 ± 13.12	15.89 ± 14.87	0.59
HF (power)	13.79 ± 13.2	8.46 ± 8.81	0.16
LF (nu)	63.97 ± 18.3	67.56 ± 16.65	0.54
HF (nu)	36.16 ± 18.21	29.56 ± 13.61	0.22
LF/HF	1.4 ± 1.14	1.78 ± 1.15	0.32

No significant difference in the HRV parameters (both sympathetic and parasympathetic function) was observed between bleeders and non-bleeders in the upright position.

Discussion

Autonomic dysfunction is commonly noted in patients with chronic liver disease and increases with worsening liver dysfunction (10, 59).

Autonomic dysfunction has been correlated with mortality (9, 10, 13, 59) and variceal bleed (11) in patients with cirrhosis liver. Rangari et al (53), in the only study on heart rate variability in patients with liver cirrhosis from India, used time domain methods for HRV analysis. They showed that four out of the five time domain measures were abnormal in patients with liver cirrhosis.

The present study, based on both time domain (Mean R-R & SDNN) and frequency domain measures (LF & HF) of HRV analysis, has shown that autonomic function is significantly impaired in patients with cirrhosis of the liver.

In the supine position, cirrhotics had a significant increase in their sympathetic tone (compared to controls) as evidenced by an increase in the LF (n.u). They also had a reduced heart rate variability (reduced SDNN) implying reduced parasympathetic tone. Thus, at rest in supine position, cirrhotics have a relatively increased sympathetic and a decreased parasympathetic tone. This is consistent with the observation of a hyperdynamic circulation in cirrhosis liver with an activation of the RAAS (Renin-Angiotensin-Aldosterone system) and the SNS (Sympathetic nervous system) pathway (20). This also correlates with other studies on heart rate variability in patients with cirrhosis of the liver (60, 62).

As the cirrhotic patient assumes an upright position, the compensatory changes in the autonomic function in the form of a raise in sympathetic and a relative fall in parasympathetic tone (observed in controls), is not evident.

In a similar study by Laffi et al (37), on the effect of passive head tilting in patients with liver cirrhosis, it was observed that there was no difference in the power spectral analysis data (LF and HF) between controls and cirrhotics in the supine position. However, cirrhotics had a higher plasma norepinephrine levels in the supine position. On passive head tilting, observations similar to the present study were

made.

With worsening liver dysfunction (CP class A vs B/C), an increase in the LF (n.u) was observed in the supine position, implying an increase in the sympathetic tone. This is consistent with the observation that the splanchnic arteriolar vasodilatation and hyperdynamic circulation seen in advanced cirrhosis leads to the activation of the SNS and the RAAS pathway (20).

Varghese et al (11) assessed the ANS function in 2 groups of cirrhotics: those with and without a history of variceal bleed. It was found that AD was significantly present in cirrhotics with a history of variceal bleed. However, assessment of the ANS function was using the standard tests (39) and heart rate variability analysis was not employed. In our study, there was no significant difference in the HRV parameters between variceal bleeders and non-bleeders.

Presence of autonomic neuropathy has been regarded as a poor prognostic indicator with increased mortality noted in patients with AD (10, 12, 59). In the present study, however, there was no difference in the HRV parameters between survivors & non-survivors and AD did not predict mortality in the study group.

The causes of death in our study group were- Variceal bleed: 5 patients; Spontaneous Bacterial Peritonitis (SBP): 4 patients; Hepatoma: 2 patients and Leptospirosis with renal failure: 1 patient. Variceal bleed was the most common cause of death and as mentioned above, there was no difference in the HRV parameters between bleeders & non-bleeders in our study. Hepatitis B was the most common etiology in our study group and it is known that hepatocellular carcinoma can occur in such patients at a less advanced stage of chronic liver disease (66). Leptospirosis, endemic in Chennai city (68), was the cause of death in one patient. SBP, a complication of advanced chronic liver disease was the cause of death in only 4 out of the 12 patients. Hence, not all deaths in the study group were related to end stage liver disease, which could explain the reason for no significant difference in the HRV parameters between survivors and non-survivors. Also, our study group consisted of only 38 patients with a follow up period of one year. Further studies with a larger study group and with a longer

duration of follow up might probably yield a positive correlation between autonomic dysfunction and mortality in patients with chronic liver disease.

Summary

Figure 4: Parasympathetic and Sympathetic tone in controls and patients with cirrhosis (Child's class A/B/C)

	Parasympathetic tone	Sympathetic tone
Controls supine position		
Controls upright position		
Cirrhotics supine position		
Cirrhotics upright position		
Child class A vs B/C		

Controls in the supine position have a balanced sympathetic and parasympathetic tone. On assuming an upright posture, there is a physiological increase in the sympathetic and a decrease in the parasympathetic tone in the control group.

Cirrhotics, in the supine position, have a relatively increased sympathetic compared to the parasympathetic tone. On assuming an upright posture, the physiological increase in the sympathetic and a decrease in the parasympathetic tone (observed in controls), is not seen in cirrhotics.

With worsening liver dysfunction (Child class A vs B/C), there is an increase in the sympathetic tone.

Conclusion

HRV analysis is a simple, noninvasive test to assess the cardiovascular autonomic function in patients with chronic liver disease .

Patients with liver cirrhosis have significantly reduced heart rate variability (decreased parasympathetic activity) and an increased sympathetic tone in supine posture.

Cirrhotics have an abnormal homeostatic response to standing with no increase in sympathetic tone in the upright posture.

With worsening Child Pugh class (A vs B/C), there is an increase in autonomic dysfunction, with an increased activity of the sympathetic component.

There were no significant differences in HRV parameters between bleeders versus non-bleeders and survivors versus non-survivors.

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Proforma

Name: Age/Sex:

Address/ Contact No: MGE No:

Etiology: HBV/HCV/ Wilson's disease/ Others (Specify) Child Pugh Score:

Clinical features:

Symptoms	Yes/No	Symptoms	Yes/No
Ascites/ Pedal Edema		Altered sensorium	
Pain Abdomen		GI bleed	
Jaundice		Coagulopathy	
Oliguria		Others (Specify)	
Autonomic symptoms (postural dizziness)		Co-morbidity (DM, HTN, CAD, others specify)	

Examination:

Jaundice/ Pallor/ Pedal edema/ Spider naevi

Vital Signs: PR: BP: Temp:

Abdomen: Liver: Spleen: Ascites:

CVS/RS:

CNS: Asterixis/ Hep. Encephalopathy:

Investigations

Hb: B Urea:

TC: S Creatinine:

DC: S Electrolytes:

ESR: B Sugar:

Platelets : PT (T/C):

MCV/MCH/MCHC :

INR:

S Bilirubin (T/D):

USG Abdomen/ PV Doppler:

AST/ALT

SAP/ GGT:

CXR:

Alb/ Globulin:

Ascitic fluid cell count:

OGD: Esophageal varices:

Gastric varices:

HRV analysis:

Mean RR interval:

SDNN:

LF (power):

HF (power):

LF (n.u)

HF (n.u)

LF/ HF ratio:

Follow Up:

Alive/ Dead:

Cause of death (when applicable);

APPENDIX D

Consent Form

I, Mr. _____, understand that **Dr. xxxxxxx**, a postgraduate student in Stanley medical college and hospital, Chennai, is doing a study on heart rate variability. I am given to understand that these tests will assess the functioning of my heart and blood vessels and the nerves regulating them. These tests are simple; involve taking ECGs, blood pressure and respiratory movements in lying and standing position. They do not involve injections or taking any medicines and are risk free. I have been familiarized with the testing procedures. I am participating in this study willingly. I have not been forced to do so. I have also been told clearly that I could withdraw from this study without any prejudice.

Date :

Signature :

Figure 4: Electrophysiology Lab with HRV recording software

Figure 5: Computerized digital recording of HRV